

d.) Remarks

Any inquiry concerning this communication or earlier communications from the applicant should be directed to Chuan Li whose telephone number is (858) 361-7231. The applicant can normally be reached from 9:00 to 5:00 pacific standard time.

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Cloning and Expressing an Oncogenic Kinase by DetoxC Strain from Expression Technologies Inc.

Cloning and expressing recombinant proteins are part of our R&D program. We cloned and expressed many proteins without any problem. However we encountered problems when we attempted to clone an oncogenic kinase into a lac operon based commercial expression vector. It is a TA based PCR cloning system that recommends using a commercial strain of E.coli from Invitrogen (which had worked well for me previously) for cloning and expression. I screened approximately 200 colonies, 80% of which had a backwards insert, 10% had a forward insert, 10% were self relegated vector. The 10% that go in forward all have mutations, even though I used a high fidelity DNA polymerase and its PCR product is only about 1000 bp.

Using a different strategy, I ligated the non-mutated regions from two of the mutated clones of known sequence, which would theoretically result in a complete vector containing the correct sequence. I transfected the resulting ligation product into the same strain of E. coli as before. I analyzed 20 clones by this method; in 19 of 20 clones the insert went in backwards, and the one that was in the correct orientation had a large deletion. At this point I concluded that expression of the oncogenic kinase was toxic to the host cell and that lacking correct clone was result of negative selection of the host cells against plasmids containing the correct insert.

We licensed and purchased detoxification E.coli strains from Expression Technologies Inc. (Exptec). Using Exptec's DetoxC strain and using the same restriction enzyme based strategy as before; I analyzed 9 clones, 8 of which had the correct sequence. One of the correct clones was expressed in the DetoxC strain. The oncogenic kinase was highly expressed. The purified protein has enzyme activity similar to that of a commercial source of the same protein.

In summary, we successfully cloned and expressed a functional oncogenic kinase by using an Exptec' detoxification bacterial strain. The same protein cannot be cloned using different strategies and a cell strain from another commercial source even though significant more clones were screened. We are pleased that our cloning and expression problems are finally solved and that Conforma can now move forward with the drug discovery program.



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